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Taste Profiling of *Centella Asiatica* by a Taste Sensor

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A taste sensor was used for organoleptic profiling and quality evaluation of *Centella asiatica* extracts and isolates on the basis of basic tastes i.e., sweet, sour, bitter, salty and *umami*. The sensor uses an array of electrodes composed of different lipid polymer membranes. The potentiometric data obtained were classified using principal component analysis (PCA) and discriminant function analysis (DFA). A good correlation was obtained between *Centella asiatica* extracts ($r>0.97$) and the salty taste, and isolates ($r>0.94$) and *the umami* taste. Similar results were obtained from the DFA method.

1. Introduction

The usage of herbal products is currently on the rise because of their vast therapeutic potential. To utilize the full potential of herbal products, methods of standardization are required to check their authenticity, quality and purity. Misidentification and adulteration could have serious consequences. New evidence is validating the usefulness of herbal medicines, including the substantiation of synergism, where the clinical effect is often more efficacious, less toxic, or both, than the effects of isolated ingredients.

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Current attempts at standardization have been largely confined to reductionist approaches based in the identification and quantification of one or two constituents believed to be the 'active ingredients' and perhaps a single mechanism of action. These approaches do not fulfil the observed value of synergism and are only applicable to those materials that have been very well studied in the laboratory.[1]

The current work will present an alternative approach that allows highly precise standardisation both chemically and biologically without reliance on a single active ingredient or mechanism of action. This approach can also find application in the quality control of mixtures of plant extracts.

2. Material and Methods

2.1 *Materials*

Poly (vinyl chloride) (PVC) used for membrane preparation was selectophore grade (Fluka Chemika, Switzerland). The lipids used were obtained from the following sources: oleic acid (OA) and decyl alcohol (DA) (~99.99%) from Fluka Chemika, Switzerland; trioctyl methylammonium chloride (TOMA) and dioctyl phosphate (DOP) from Tokyo Chemical Industry, Japan; dioctyl phenylphosphate (DOPP) (plasticizer) (95%), oleyl amine (OAm) from Aldrich Chemical. Chemicals and solvents used in the analytical procedure were all of analytical grade.

Centella asiatica of different species and its pure isolates: asiatic acid, madicassic acid, asiaticoside and madecassoside, were provided by the Forest Research Institute of Malaysia (FRIM).

2.2 *Methanol extract*

Centella asiatica herb was dried at 50°C and ground. The powdered plant (70 gm) was extracted in a soxhlet extractor with methanol for 48 h, filtered and evaporated to dryness under vacuum at 40°C in a rotary evaporator to yield a dried extract. From the crude dried extract, 0.1%, 0.03%, 0.01%, 0.003% and 0.001% solutions were prepared.

2.3 *Phytochemical screening*

Crude extract, asiatic acid, madecassic acid, asiaticoside and madecassoside were dissolved in methanol and spotted on TLC plate (silica gel Merck $60F_{245}$ 10×20 cm, with layer thickness of 0.25 mm). The plate was developed in a solvent system chloroform / glacial acetic acid / methanol / water in the 100 : 40 : 16 : 8 ratio.[2] Spots were detected under visible and UV-365 nm after spraying anisoldehyde-sulfuric acid reagent on the plate and heating at 100°C for 10 min. Rf values of asiatic acid, madecassic acid, asiaticoside and madecassoside were 88, 79, 21 and 12, respectively.

2.3.1 *Preparation of crude extracts for measurements*

Powder *Centella asiatica* (2 g) for each of the samples CA01 to CA12 was refluxed

with 40 ml water for 5 min. The extracts were filtered and evaporated to dryness with a rotary evaporator. Methanol extraction of the samples CA01 to CA12 was done by refluxing the 2 g powder samples. The solutions were filtered and evaporated to dryness. Water and methanol extracts were dissolved in distilled water to obtain a quantity of 50 ml.

2.3.2 *Isolation of TLC spots from crude extract*

Crude drug extract (100 mg) was dissolved in 2 ml methanol and run on a TLC plate with mobile phase. The plates were scraped at the areas having the same hRf values as asiatic acid, madecassic acid, asiaticoside and madecassoside. Each fraction was extracted with 20 ml methanol three times and the pooled extract was evaporated to dryness at 40°C. The dried components were dissolved in distilled water to obtain a quantity of 25 ml.

Four standard solutions of asiatic acid, madecassic acid, asiaticoside and madecassoside were prepared by dissolving 0.2 mg of each in 25 ml distilled water.

2.4 *Taste sensor system*

The samples were analyzed using an 8-channel high-impedance multiinterface meter from Fylde Scientific U.K. The lipid materials used in the membrane preparation and their arrangement are similar to those reported,[3] with an addition of channel No. 8 (DOP:TOMA=9:1). To prevent interferences due to different charges,[4] negatively charged electrodes (Channel 1, 2, 3 and 8) and positively charged electrodes (Channel 4, 5, 6 and 7) were separated. The potential difference between the electrodes and reference electrode (Ag/AgCl with saturated KCl) were measured in two separate beakers.

Quinine 0.1, 0.3, 1, 3 and 10 mM, sodium chloride 3, 10, 30, 100, 300 and 1000 mM, hydrochloric acid 1, 3, 10, 30 and 100 mM, sucrose 30, 100, 300 and 1000 mM and monosodium glutamate 1, 3, 10, 30 and 100 mM were prepared for the five basic tastes of bitter, salty, sour, sweet and *umami*, respectively. The lowest concentrations nearly corresponded to the threshold value detectable by humans.

Prior to use, the electrodes were conditioned in 1 mM KCl for one hour followed by washing with deionized distilled water. After washing, the electrodes were left in the sample for one minute before measurements were monitored for another min. Each sample was measured three times. Electrodes were thoroughly rinsed with distilled water prior to the next measurement. The potential difference was taken as the difference between a sample and 1 mM KCl. Electrodes were suspended in air when not in use.

3. Result and Discussion

Potentiometric measurements in 1 mM KCl were conducted at least for 3 h to observe the electrode performance prior to further analysis. Standard deviations were less than 1% of each electrode in different concentrations. for example, for the membrane of DOP (dioctyl phosphate, channel 3), the standard deviations were 0.73, 0.73, 0.91, 0.48 and 0.78 mV for 0.1, 0.3, 1, 3 and 10 mM quinine solutions, respectively.[5]

Figure 1(a) shows the electrical potential pattern response of *Centella asiatica* methanol extracts. The electrical potential data of extracts was analyzed using principal component analysis (PCA). The contribution rates of the original data to PC1 and PC2 are 74.13% and 12.44%, respectively. The plot of PC1 vs. log concentration is shown in the Fig. 1(b). The PC1 value changes with concentration and therefore could be used for *Centella asiatica* extract evaluation. The range of detection obtained was 0.001% to 0.1% of the dried methanol extract.

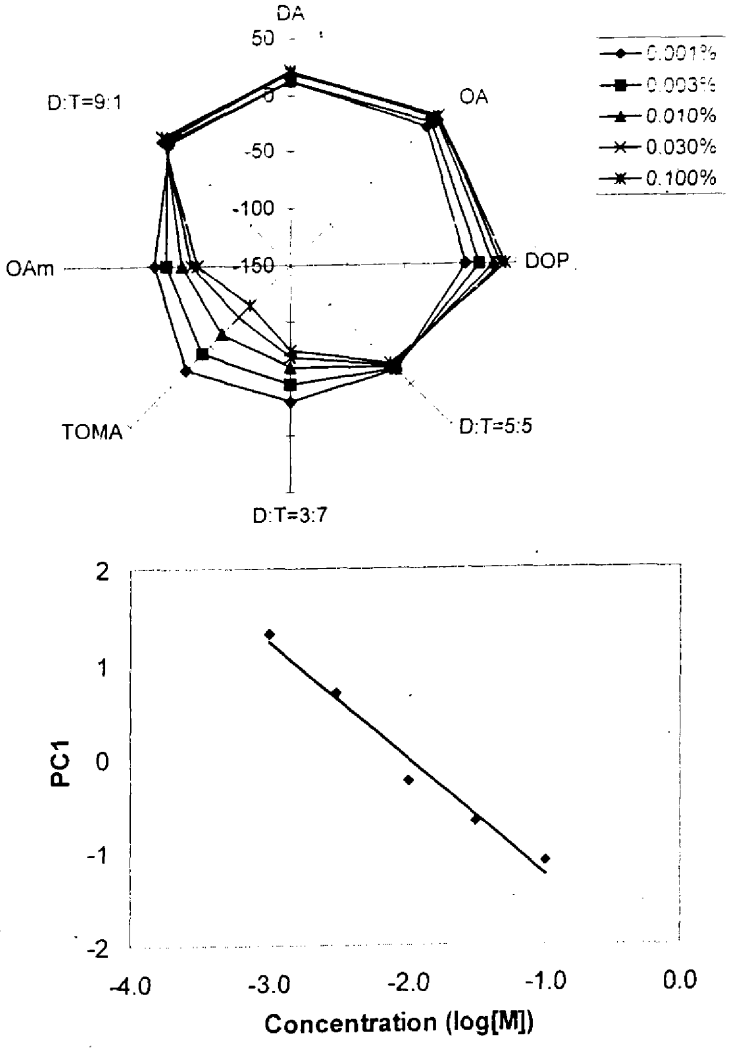

Fig. 1. (a) Electrode potential pattern and (b) PCA of *Centella asiatica* extracts at different concentration.

Figures 2(a) and (b) show the response pattern of water extracts and methanol extracts for different species of *Centella asiatica*, respectively. Both extracts contain a mixture of triterpenoid glycosides, including asiaticoside, medecassoside, vellerin, a bitter principle tannin, alkaloid, volatile oil, and pectin,[5] however, their quantities are different. This is reflected by their slightly different visual response patterns. Different species of *Centella asiatica* for both extracts give similar response patterns but with some difference in mV intensities.

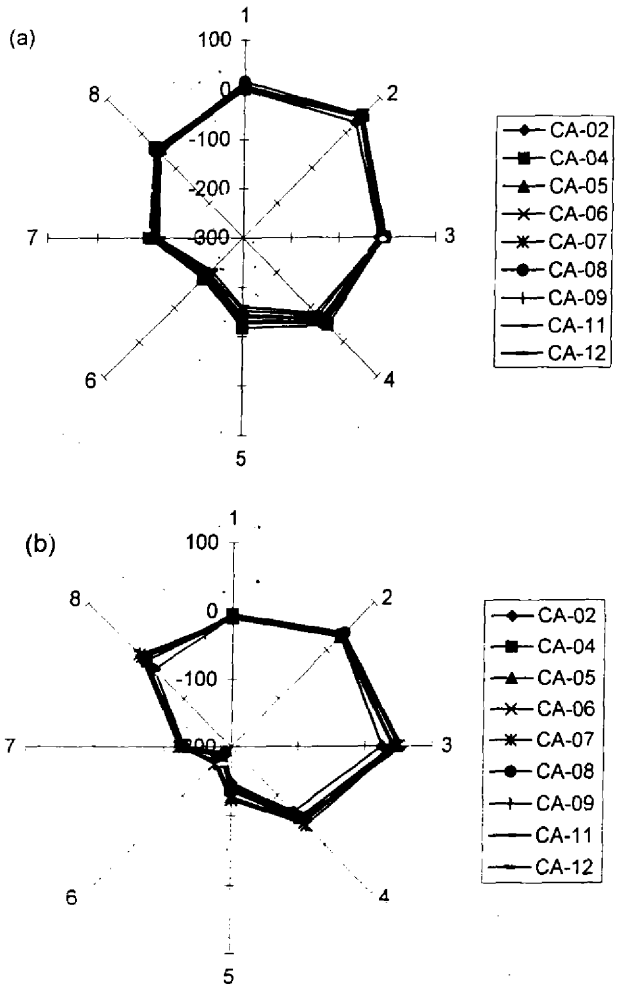

Fig. 2. Response potential pattern of *Centella asiatica* (a) water extracts and (b) methanol extracts.

Figure 3(a) shows the response potential of *Centella asiatica* pure active compounds, asiatic acid, madecassic acid, asiaticoside and madecassoside, and Fig. 3(b) shows the response pattern of the above compounds, which were scraped off from the TLC plate at their respective Rf values. The sensor was found to be sensitive to pure compounds (triterpenoids). The electrode potentials of DOP and DA decrease and TOMA, OAm, D:T=5:5 and D:T=3:7 negative decreases with increasing concentrations of asiatic acid and madecassic acid.

Tastes of different species of *Centella asiatica* extracts are similar and difficult to differentiate orally. This experiment attempts to classify *Centella asiatica* extracts on the basis of their basic tastes, by means of a taste sensor.[6] The data from the experiment were analyzed using a multivariate data analysis method, namely, PCA and discriminant function analysis (DFA).

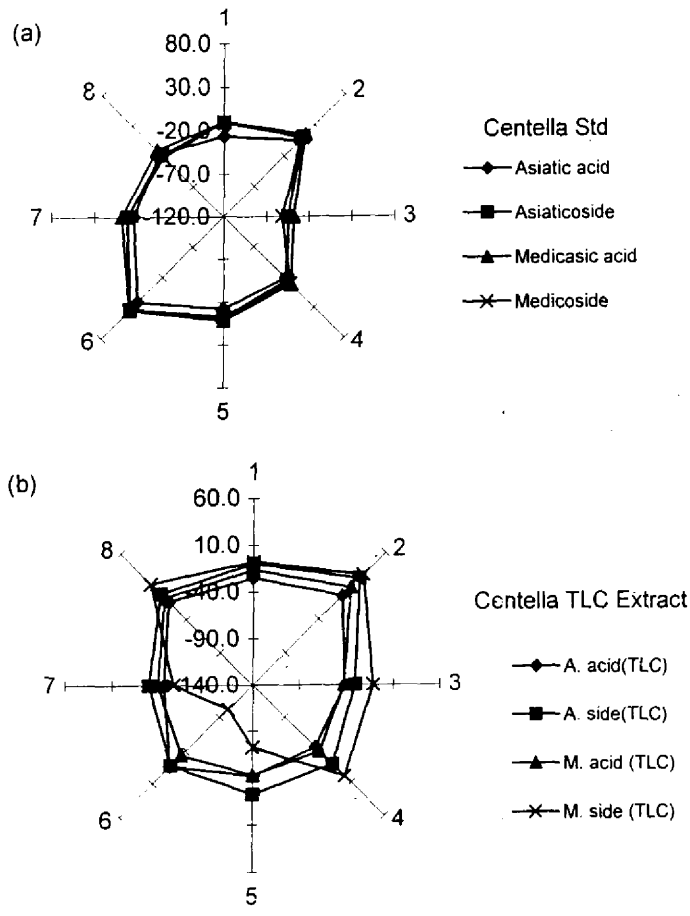

Fig. 3. Response patterns of isolates from *Centella asiatica*. (a) Pure isolates, (b) separated from TLC plates.

PCA is a statistical technique that linearly transforms an original set of variables into a substantially smaller set of uncorrelated variables that represents most of the information in the original set of variables.[7] Its goal is to reduce the dimensionality of the original data set without losing information on the total variation of the original data set. The percentage of the data variance contained in each principal component is given by the corresponding eigenvalues.

Figure 4 shows the score plot of principal components 1 and 2. The first and second principal components contain 92% of the total data variance, which means that both PC1 and PC2 characterized the pattern shown. For the classification of taste using PCA, first, the group centroids of each taste group are obtained from the mean of PC1 and mean of PC2. Then, on the basis of the PC scores of each sample, the distances to the five group centroids were calculated. Each sample was assigned to a particular taste based on the shortest distance between their centroids. In this case, correct classification by PC1 and PC2 is 76%. Due to the high percentage (i.e., 24%) of misclassification, PCA is not suitable for classification. In this case, it may be expected that DFA could perform better classification, since DFA constructs functions to help classify the data whereas PCA constructs functions to account for the variance of the data as a whole.[8]

Discriminant analysis attempts to classify samples into known taste groups by constructing a linear relationship of sensor outputs.[8] In order to classify samples using DA, a calibration model is needed, which will classify the data set best.[9] The calibration model will be used to recognize further unknown samples.

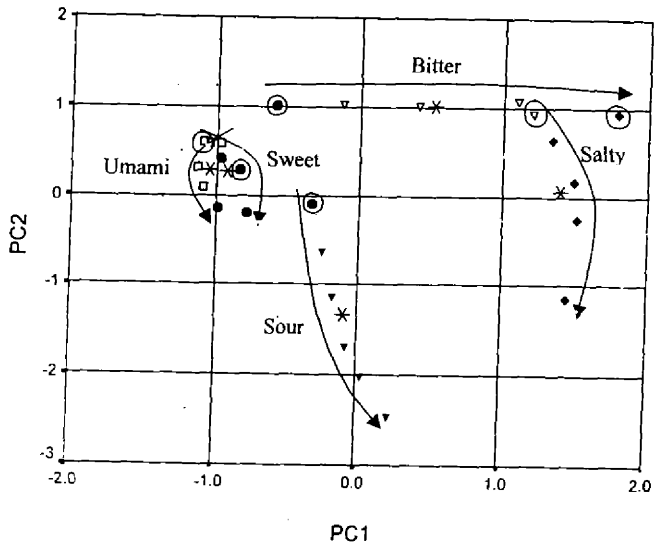

Fig. 4. Classification of five basic tastes by PC1 and PC2 functions. The circles indicate the misclassifications of taste. * Group centroids, ▽ Bitter, ◆ Salty, ▼ Sour, ● Sweet and □ *Umami*.

Twenty-five data from eight electrode readings from bitter, salty, sour, sweet and *umami* tastes were used to extract the classification model. A total of four canonical discriminant functions were extracted for the five basic tastes. First and second discriminant functions cumulate 99.9% of the total variance. Figure 5 shows that the first discriminant function reflects the group difference between sourness (negative value of the plot) and sweetness and *umami* (positive value), and the second discriminant function reflects the group difference between saltiness (negative value) and bitterness (positive value). Classification using the first discriminant function is shown in Fig. 5. Notice that a plot with discriminant function scores 1 and 2 is used only as a reference. In this case, one data from a sweet taste was misclassified as an *umami* taste, which left the total number of sweet samples of three, as shown in Table 1. Numbers refer to the total number of samples taken for the analysis. In this case, misclassification is 4% from the twenty-five data taken. A correct classification was obtained when two discriminant functions were used. The previously 4% misclassified case is now correctly classified.

After obtaining the classification rule from 25 samples, this rule was used to classify water extracts, methanol extracts and isolates (pure standards and from TLC) of *Centella asiatica*. First and second discriminant functions were used for the classification of *Centella asiatica* taste as shown in Fig. 6. Water and methanol extracts are closest to the group centroids of the salty taste and therefore were classified as salty.

The potentiometric data obtained from the taste sensor were compared with the five basic tastes using a normalization procedure to extract the property of the response

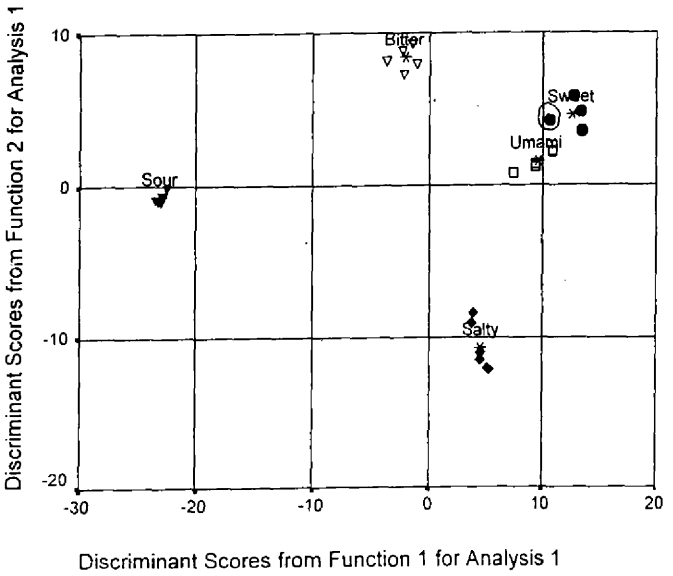

Fig. 5. Classification of basic taste using first discriminant function. The circles show misclassification. * Group centroids, ▽ Bitter, ◆ Salty, ▼ Sour, ● Sweet and □ *Umami*.

Table 1
Cross-validation of taste and classification with discriminant factor 1.

	Classification by discrimination Function 1					
Category of Taste	Bitter	Salty	Sour	Sweet	Umami	Total
Bitter	5					5
Salty		6				6
Sour			5			5
Sweet				3	1	4
Umami					5	5
Total	5	6	5	3	6	25

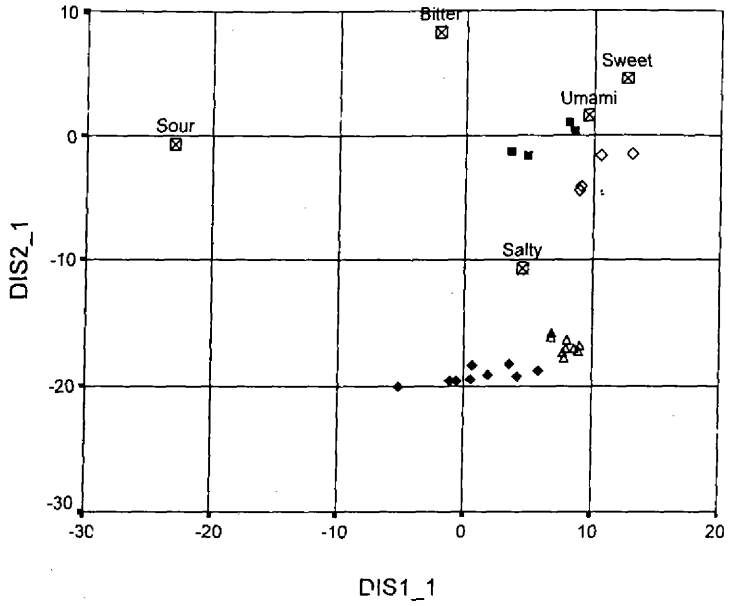

Fig. 6. Classification of *Centella asiatica* by discriminant function scores 1 and 2. ⊠ Group centroids, ◆ methanol extracts, △ water extracts, ■ triterpenic acids, ◇ triterpenic glycosides.

pattern.[6] The normalized response patterns of methanol extracts were found to have a high correlation with the salty ($r>0.97$) and sour ($r>0.86$) tastes similar to the water extracts with the salty ($r>0.96$) and sour ($r>0.84$) tastes. However, for asiatic acid, madecassic acid, asiaticoside and madecassoside, the response patterns have a high correlation ($r>0.93$) with *umami* taste. A similar observation was found from the discrimination analysis, i.e., water and methanol are extracted having a salty taste and isolated having an *umami* taste.

4. Conclusion

The feasibility of using a taste sensor to classify CA extracts has been discussed in this study. The sensor was able to correctly classify basic tastes using the DFA method. The sensor was then used for the classification of *Centella asiatica* extracts and isolates. Good correlations were found between the potentiometric patterns of *Centella asiatica* extracts and the salty taste and between isolates and the *umami* taste. A similar observation was made when the DFA method was employed.

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